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ORIGINAL ARTICLE

Contrast-enhanced Ultrasound for Detection of Traumatic Splenic Bleeding in a Canine Model During Hemorrhagic Shock and Resuscitation

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Background: Contrast-enhanced ultrasound (CEUS) is a highly specific and sensitive method for the detection of abdominal injury. We assessed the value of CEUS for real-time monitoring of splenic trauma and detection of bleeding in a canine model of splenic injury during hemorrhagic shock and resuscitation.
Methods: Forty Grades III and IV traumatic splenic lesions were created in 15 mongrel dogs. Hemorrhagic shock was induced by exsanguination via the left femoral artery. Animals in shock were then resuscitated with 6% hydroxyethyl starch. CEUS was performed continuously to investigate the imaging characteristics of splenic trauma and to monitor changes in bleeding.
Results: Prior to induction of hemorrhagic shock, CEUS revealed active bleeding in 36 of 40 traumatic lesions (90.0%), either as contrast medium extravasation or pooling, both in the spleen and outside the capsule. During the shock period, no traumatic lesion had active bleeding. CEUS revealed that tiny branches of splenic arteries decreased in number and became thinner. The traumatic lesions appeared as nonenhanced areas with poorly defined boundaries. After fluid resuscitation, rebleeding occurred in 30 traumatic lesions, and 28 (93.3%) of these were detectable by CEUS.

Conflicts of interest: The authors have no conflicts of interest relevant to this article.

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Conclusions: CEUS allowed real-time monitoring of splenic trauma and detection of bleeding and rebleeding in hemodynamically unstable dogs, prior to and after fluid resuscitation. This suggests that CEUS might be used in clinical intensive care for patients with traumatic hemorrhagic shock.

Introduction

The spleen is the abdominal organ most vulnerable to traumatic injury [1]. Splenic rupture, occurring in 20—46% of these cases, can cause severe internal bleeding and even hemorrhagic (hypovolemic) shock, the leading cause of early death of trauma patients [2]. Monitoring for active bleeding is imperative.

For many years, ultrasound has been the first-line diagnostic tool for abdominal organ trauma, allowing for repeated examinations of hemodynamically unstable patients [3], but is less sensitive than computed tomography (CT) for evaluating splenic parenchymal lesions. The technological successor of ultrasound is contrast-enhanced ultrasound (CEUS). Together with the newly developed ultrasound contrast agents, CEUS allows detection and monitoring of traumatic lesions and active bleeding in real time [4—6]. Tissue perfusion and clinical treatment response can be continuously followed throughout the day [7]. Moreover, compared to CT, CEUS is more suitable for bedside examination and intensive care [8].

The condition of patients with traumatic splenic bleeding can change rapidly and hemorrhagic shock may develop suddenly [2]. In such cases the risk of death increases by 1% for every 3-minute delay of treatment [9]. Because CEUS immediately detects the bleeding site and the type, size, and location of the lesion, the time needed to identify and control bleeding (surgically or conservatively) is reduced [5,10] and the patient is rapidly stabilized.

Although there are recent reports from major trauma centers that CEUS is increasingly used for patients with active internal bleeding [5,10], studies that have assessed its utility have mostly focused on hemodynamically stable animals or patients; few have used a splenic injury model of hemorrhagic shock. In the current study, we used CEUS to investigate the imaging characteristics of splenic trauma in a canine model of splenic trauma with hemorrhagic shock, prior to and after fluid resuscitation.

Methods

Animals

All animal experiments conformed to the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were conducted in accordance with a protocol approved by the Institutional Animal Care and Use Committee.

Seventeen 2—3-year-old healthy male mongrel canines, weighing 16.5 $\pm$ 1.5 kg, were used in the current study. All animals underwent a 3-day adaptation period. The animals were maintained in cages with a 12-hour light—dark cycle, dog chow was provided twice daily, and water could be accessed freely. Dogs were considered healthy after clinical examination and a normal erythrocyte count. Prior to surgery, the animals were fasted for 12 hours with free access to water.

Establishing the animal model

The animals were anesthetized by injection of 3% pentobarbital sodium (30 mg/kg body weight; Sinoreagent, China) via an intravenous catheter placed in a forelimb vein. After successful anesthesia was achieved, the dogs underwent endotracheal intubation with continuous machine-assisted ventilation. Anesthesia was maintained throughout the procedures via intermittent intravenous injection of 3% pentobarbital sodium. Systemic heparinization was achieved by intravenously injecting heparin (300 IU/kg, Baiyun Pharmaceutical, Guangzhou, China).

The same operator created all of the traumas. A ventral midline incision was made under aseptic conditions to open the abdominal cavity and fully expose the spleen. Using the blunt handle of a small surgical knife, a cross-shaped wound with relatively rough edges (transverse diameter 2.0—3.0 cm, longitudinal diameter 1.0—2.0 cm) was created in the spleen. The wound did not involve blood vessels at the splenic hilum. Based on classification criteria established by the American Association for the Surgery of Trauma [11], the trauma mimicked Grades III and IV splenic injury. Forty Grades III and IV traumatic splenic lesions were created in 15 mongrel dogs, including 17 for Grade III and 23 for Grade IV. Persistent active bleeding was used as the criterion for successful modeling; otherwise, the animals were excluded from the study.

Hemorrhagic shock was induced using the improved Wiggers method [12—14]. Exsanguination via the left femoral artery at 2.0—2.5 mL kg^{-1} min^{-1} was performed until the mean arterial pressure was stable at 45.0 $\pm$ 0.18 mmHg for 60 minutes. Mean arterial pressure was maintained by means of bloodletting or autologous transfusion. This period was defined as the shock period.

Sixty minutes after induction of hemorrhagic shock, fluid resuscitation was initiated by intravenous infusion of 6% hydroxyethyl starch (Voluven; Fresenius Kabi, Bad Homburg, Germany) via the right femoral vein. Fluid resuscitation was performed for a period of $\leq$ 120 minutes. Successful resuscitation was defined as mean arterial pressure $>$ 80 mmHg for $\geq$ 20 minutes. This period was defined as the resuscitation period.

During the procedure, the spleen size and vital signs [i.e., mean arterial pressure (MAP), heart rate (HR), and

arterial oxygen saturation (SaO_2)] were continuously monitored.

Ultrasound imaging

The second-generation sonographic contrast agent SonoVue (Bracco, Italy) was used in this study. It is made of microbubbles (90% of microbubbles $<$ 8.0 μm, mean diameter 2.5—3.5 μm, pH 4.5—7.5) stabilized by phospholipids and filled with sulfur hexafluoride. Prior to use, lyophilized SonoVue powder was mixed with 5 mL of normal saline and then administered intravenously. Each bolus injection (0.8—1.2 mL) of the contrast agent was followed by a 5-mL saline flush. After injection of the contrast agent, sonography was immediately performed to monitor dynamic splenic injury. The interval between the two injections of the contrast agent was more than 10 minutes.

Ultrasound was performed using a Philip CX50 color Doppler ultrasound system (Philips Medical Systems, Andover, MA) with an L12-3 broadband linear ultrasound transducer (frequency 3—12 mHz). CEUS was performed using pulse-inversion harmonic imaging with a mechanical index of 0.07.

CEUS were performed at various stages of the experiment with the scan transducer on the surface of the splenic trauma. The location, shape, size, and sonographic appearance of the area of trauma were observed. All CEUS scans were performed by the same physician. Splenic trauma was defined as the presence of perfusion defects in the splenic parenchyma that were not enhanced, or were slightly enhanced. Traumatic active bleeding was defined as the presence of contrast agent spillover or uptake within the lesion and the surrounding areas. Two experienced radiologists analyzed the CEUS images to make a diagnosis.

Statistical analyses

Statistical analyses were performed using SPSS version 13.0 software (SPSS Inc., Chicago, IL, USA). Numeral data were expressed as mean ± standard deviation. Means between two groups were compared using Student t test. A p value $<$ 0.05 was considered statistically significant.

Results

Creation of an animal model

Seventeen dogs underwent the surgical procedure. Fifteen dogs survived the hemorrhagic shock period; two dogs died during the shock period and were excluded from analysis. Forty traumatic splenic lesions, Grades III and IV, were created in the 15 surviving dogs.

Comparison of physiological indices among different stages

MAP, SaO_2, and splenic length decreased whereas HR increased significantly in all animals with shock. However, MAP, SaO_2, and splenic length increased significantly after fluid resuscitation (Table 1).

Perfusion parameters of splenic parenchyma

The parenchymal perfusion parameters, including arrival time, time to peak intensity, peak intensity, and washout time were evaluated (Table 2). During the period of hemorrhagic shock, the arrival time, time to peak intensity, and washout time were significantly longer, and the peak intensity was lower, compared with these parameters prior to shock was induced. Fluid resuscitation significantly reversed all these effects. That is, fluid resuscitation shortened the arrival time, time to peak intensity, and washout time and increased the peak intensity.

CEUS imaging characteristics of splenic trauma

Prior to induction of hemorrhagic shock, the spleen was bright red in color with good elasticity (Fig. 1A). CEUS revealed active bleeding in 36 of 40 traumatic lesions (90.0%), observed as contrast medium extravasation or pooling, both in the spleen and outside the capsule (Fig. 1B).

During the shock period, the spleen significantly shrank and became wrinkled, and had a dark red color and hard texture (Fig. 2A). The traumatic lesions had no visible active bleeding. CEUS revealed that the tiny branches of splenic arteries decreased in number, became thinner, and were dead and branch-like in shape (Fig. 2B). The contrast agent accumulated into floccules at the ends of small arterial branches and was slowly diffused. The traumatic lesions appeared as nonenhanced areas with blurred boundaries, and there were no signs of active bleeding.

After fluid resuscitation, the size of the spleen was similar to that prior to hemorrhagic shock, with a slightly dark red color and recovered elasticity (Fig. 3A). Rebleeding occurred in 30 traumatic lesions. CEUS revealed that the tiny branches of splenic arteries reperfused rapidly. Splenic

Table 1 Comparison of major physiological indicators and spleen length among different shock stages.

Stage	MAP (mmHg)	HR (bpm)	SaO_2 (%)	Spleen length (cm)
Preshock	148.20 ± 18.99	113.07 ± 22.89	97 ± 0.92	27.47 ± 1.50
Shock	49.47 ± 5.60[a]	166.47 ± 18.20[a]	93 ± 2.16[a]	5.80 ± 1.14[a]
Resuscitation[b]	136.40 ± 23.11[c]	159.47 ± 31.58[a]	95.53 ± 1.73[a,c]	23.87 ± 2.95[a,c]

HR = heart rate in beats per minute; MAP = mean arterial pressure; SaO_2 = oxygen saturation of available hemoglobin.
[a] Compared with the preshock stage, $p < 0.05$.
[b] Resuscitation stage: 120 minutes postresuscitation.
[c] Compared with the shock stage, $p < 0.05$.

Table 2 Parenchyma perfusion parameters at different stages of induced splenic trauma.

	Arrival time (s)	Time to peak intensity (s)	Peak intensity (dB)	Washout time (min)
Prior to hemorrhagic shock	4.11 ± 0.23	7.63 ± 0.77	187.47 ± 1.51	9.63 ± 0.41
During hemorrhagic shock	7.39 ± 0.68[a]	15.14 ± 1.81[a]	113.57 ± 2.14[a]	113.57 ± 2.14[a]
After fluid resuscitation	4.29 ± 0.34[b]	4.29 ± 0.34[b]	180.95 ± 2.88[b]	10.21 ± 0.13[b]

[a] $p < 0.01$ compared with prior to hemorrhagic shock.
[b] $p < 0.01$ compared with during hemorrhagic shock.

parenchymal perfusion was enhanced heterogeneously. Twenty-eight of the 30 lesions (93.33%) showed abnormal enhancement, suggesting the occurrence of rebleeding (Fig. 3B).

Discussion

In the current study, we observed that when splenic parenchymal injury with active bleeding occurred, blood perfusion in the injured area was altered and the radial pattern of contrast enhancement of the splenic artery, from the splenic hilum to the splenic surface, was interrupted. Prior to hemorrhagic shock, we used CEUS to identify sites of active bleeding, because there is a variation in perfusion between the lesion and its surrounding tissues. As in our previous studies [15], we found that the variability in perfusion was caused by extravasation of the intravascular contrast agents, and that the active bleeding sites presented various shapes depending on the location, velocity, and quantity of perfusate.

If hemorrhagic shock is not managed within a certain time the body's effective circulating blood volume will be drastically reduced, resulting in insufficient tissue perfusion. In the current study, after induction of hemorrhagic shock, MAP and SaO_2 significantly decreased, and HR significantly increased. In dogs, the splenic capsule contains muscle; contraction of the muscle occurs to increase blood volume in response to stress [16,17]. During this stage, there was obvious shrinkage in the spleens of all the dogs.

Bleeding from a ruptured spleen has a tendency to stop spontaneously. This may be because of shrinkage of the vascular stump and spleen occurs after splenic rupture, and because approximately one third of the body's platelets are in the spleen, blood clots may block the blood vessels. When shock occurs, the splenic artery is highly sensitive to sympathetic nerve stimulation, which leads to vasoconstriction and a reduction of splenic blood flow [18]. These changes in blood vessels suggest that CEUS enhancement will be greatly reduced after hemorrhagic shock, even if there is no damage to blood vessels or the splenic parenchyma. In the current study, CEUS revealed no signs of active bleeding during hemorrhagic shock. Although vasoconstriction and a reduction of splenic blood flow can help predict the occurrence of hemorrhagic shock, it may also affect the evaluation of traumatic lesions by CEUS because it can lead to a reduction in the contrast between the traumatic lesion and the surrounding splenic tissue.

Antishock measures are mandatory in patients with traumatic hemorrhagic shock, including large-volume fluid resuscitation and blood transfusion [19,20]. Although fluid resuscitation increases the effective circulating blood volume, it also reduces the body's blood clotting function. Once rebleeding begins, life-threatening irreversible hemorrhagic shock can occur. In our experiments, rebleeding occurred in 30 traumatic lesions, perhaps because of one or a combination of factors [21,22]. First, rapid fluid infusion reduced blood viscosity and accelerated blood flow—this resulted in the fragmentation or washout of the thrombi formed. Second, elevated blood pressure caused the relief

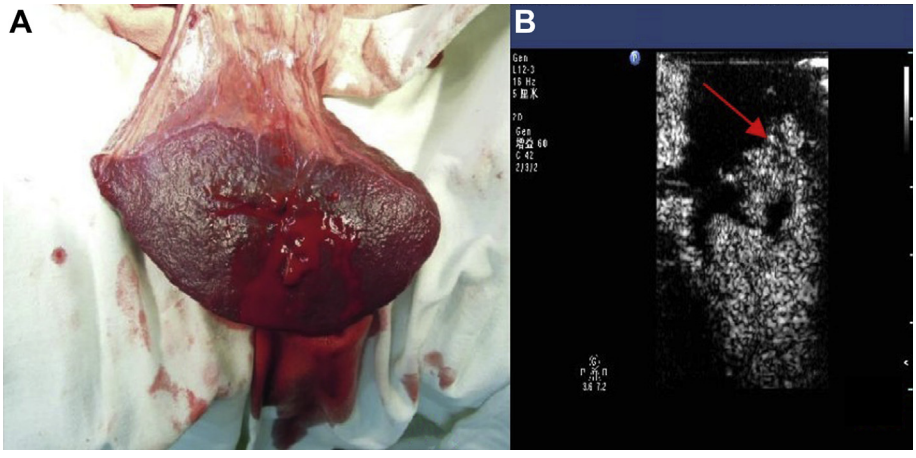

Fig. 1 Images of the spleen from a dog prior to induction of shock. (A) Gross appearance of the spleen. (B) Contrast-enhanced ultrasound of the spleen indicating the presence of active bleeding, where contrast medium extravasation and pooling (red arrow) were observed.

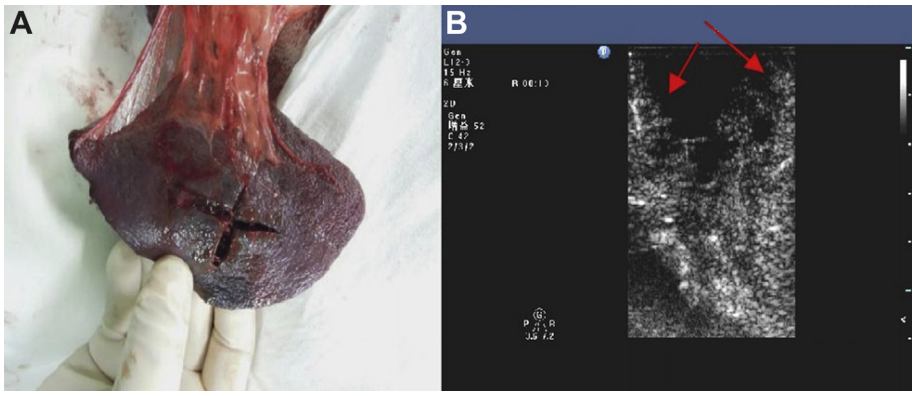

Fig. 2 Images of the spleen from a dog in traumatic hemorrhagic shock. (A) Gross appearance of the spleen. (B) Contrast-enhanced ultrasound of the spleen. The splenic lesion appeared as an anechoic perfusion defect area with an unclear irregular border (red arrows), suggesting the absence of active bleeding.

of vasospasm and the dilation of blood vessels. Third, fluid resuscitation diluted coagulation factors and reduced blood clot formation.

CEUS has an advantage over CT in evaluating therapeutic effects and monitoring rebleeding during the resuscitation period because it can be used at the patient's bedside. In our study, CEUS revealed that tissue perfusion was restored and parenchymal perfusion was enhanced after fluid resuscitation, and rebleeding was detected in 93.33% of traumatic lesions (28 of 30). Two cases of rebleeding were missed, which may have been due to low arterial flow velocity.

Bleeding can compromise microcirculation and thus organ perfusion. Because contrast agent microbubbles can mimic red blood cell flow in the microcirculation, their number and transit rate in a given organ reflects the degree of tissue microcirculation [23]. The arrival time, time to peak intensity, peak intensity, and washout time, calculated from the time-intensity curve, are quantitative determinations of the hemodynamics of the regions of interest. Indeed, we found that even when vital signs were stable after bleeding, parenchyma perfusion parameters had changed. For example, we noted a decrease in arrival time, time to peak intensity, and peak intensity, and an increase in washout time, suggestive of a preshock state. One possible cause of these changes is a vasoconstrictor reflex of the splenic capsule, by which the cortical vascular bed is reduced, thus shifting blood to the medulla [24]. An additional or alternative mechanism may be adrenoceptor overstimulation by circulating catecholamines. In dogs, the spleen has been shown to contract during experimental shock, and is likely to help maintain cardiac function [17,18]. This finding suggests that changes in these parameters may be better correlated with circulating blood volume than with blood pressure.

The current study is limited in that our canine model cannot fully replicate the clinical characteristics of patients with traumatic hemorrhagic shock. In particular, ultrasound was performed directly on the surface of the spleen. Ideally, ultrasound scanning should be carried out percutaneously. In addition, some experimental manipulations, such as induction of general anesthesia and systemic heparinization prior to splenic trauma, might have influenced the physiological parameters we measured. Finally, hydroxyethyl starch is only one of many intravenous fluids used for resuscitation and the optimal resuscitation fluid remains a subject of debate.

In conclusion, the current study provides evidence that CEUS allows for real-time monitoring of splenic trauma and detection of bleeding and rebleeding in hemodynamically unstable dogs, prior to and after fluid resuscitation. Our results suggest that CEUS might be used in the medical

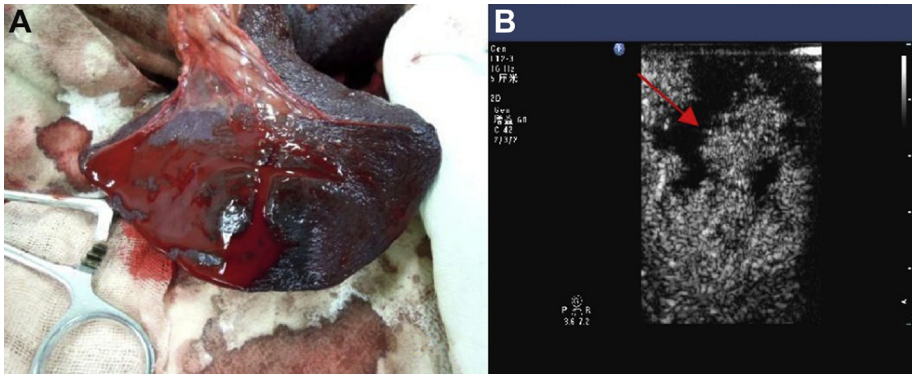

Fig. 3 Images of the spleen from a dog after fluid resuscitation. (A) Gross appearance of the spleen. (B) Contrast-enhanced ultrasound of the spleen revealing the presence of active rebleeding (red arrow).

intensive or critical care unit for patients with traumatic hemorrhagic shock.

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